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DNA Isolation

Using Human Cheek Cells

Publication No. 10196

Introduction

Learn how to isolate DNA from human cells. Yours!

Materials

Plastic drinking cup, 30-mL
Test tube, 16 × 100 mm
Test tube, 12 × 75 mm
Stopper, #2
Glass stirring rod
Tap water, 10 mL
Sodium chloride solution, 8.0%, NaCl, 20 drops
Sodium lauryl sulfate solution, 10%, $C_{12}H_{25}NaO_4S$, 20 drops [*or*]
Liquid dishwashing detergent solution, 25%, 20 drops
Ethyl alcohol, 95% denatured, CH_3CH_2OH, 6 mL
Dropping bottles, 3
Test tube rack

Safety Precautions

Ethyl alcohol is flammable and a dangerous fire risk; keep from flame and all sources of ignition. Use only clean drinking cups for this procedure. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron. Please review the relevant Material Safety Data Sheets before beginning this activity.

Preparation

1. Prepare an 8.0% w/w sodium chloride solution: Dissolve 8.0 g of sodium chloride in 92 mL of distilled water. Place solution in dropper bottle and label.
2. Prepare a 10% sodium lauryl sulfate solution: Dissolve 10.0 g of sodium lauryl sulfate in 90 mL of distilled water. Place the solution in a dropper bottle and label it. If dishwashing detergent is used, make a 25% solution by mixing 25 mL of liquid detergent concentrate with 75 mL of distilled water. Place the solution in a dropper bottle and label it.
3. Place the ethyl alcohol solution in a dropper bottle and label it.

Procedure

1. Add 1 mL (20 drops) of the 8% sodium chloride solution to the larger test tube. Set the tube aside in a test tube rack.
2. Pour 10 mL of fresh tap water or bottled water into a clean 30-mL plastic drinking cup.
3. Put the 10 mL of water in your mouth and swirl the water around for at least 30 seconds. Spit the water back into the plastic cup. (The swirling of the water washes cells from the inside of your cheeks into the water.)
4. Pour several mL of the "cheek cell" water into the test tube containing the salt solution from Step 1.

Releasing the DNA from inside the cheek cells.

5. Add 1 mL (20 drops) of the 10% sodium lauryl sulfate solution *or* 1 mL (20 drops) of the 25% liquid dishwashing detergent solution to the "cheek" mixture in the test tube.

6. Stopper the test tube and mix the contents of the tube by gently inverting the test tube several times. *Do not shake the test tube.* (The detergent removes the cell membranes from the cheek cells, releasing the DNA into the salt solution.)

Precipitate the DNA.

7. Holding the test tube at a slight angle, carefully add 5 mL of 95% ethyl alcohol down the side of the test tube so that it forms a layer over the "cheek" mixture in the test tube.
8. Hold the test tube upright for one minute and observe what happens at the interface between the ethyl alcohol and the "cheek" solution. (The clouds of white strands are the DNA. The DNA is not soluble in ethyl alcohol, so it precipitates where the two liquids meet. Soap bubbles from the "cheek" solution will get trapped in the DNA strands.)

Collect the DNA.

9. Add 1 mL (20 drops) of 95% ethyl alcohol to the smaller test tube.
10. Place a clean glass stirring rod in the test tube containing the DNA. Collect the DNA by winding it on the rod by turning the rod in one direction.
11. Carefully, remove the rod and DNA from the solution and transfer it to the smaller test tube containing 1 mL of 95% ethyl alcohol. Observe the DNA strands floating in the alcohol.

Tips

- If your tap water has any unusual properties, use bottled water for this lab.
- If your DNA yield is not sufficient for spooling, try the following:

  1—Rinse your mouth more violently and for a longer period of time.

  2—The action of the detergent in Step 5 can be enhanced by placing the test tube in a water bath at 55 °C. This enhances the action of the detergent and also denatures enzymes that might damage DNA.

  3—The alcohol used in Step 7 might be more effective if it is made ice-cold in an ice bath.

Discussion

The steps in this laboratory procedure teach a great deal about the properties of cells, cell membranes, and deoxyribonucleic acid (DNA) itself.

The collection of cheek cells from the inside of the mouth highlights the nature of body tissue. Dead cells are continually being sloughed off on both the inside and outside of the body. Recently-sloughed cells still contain their nucleus and their DNA genetic material. This DNA can be collected and if in a forensics situation, analyzed and traced to a specific individual.

Detergents solubilize and break down the lipids and proteins that form the primary cell membrane and disrupt the bonds that hold the membrane together. The cell contents, including the nucleus, are thus released and become available for further treatment or isolation. Sodium lauryl sulfate is an active ingredient in detergents.

The final step requires the alcohol. The solubilized DNA comes in contact with the alcohol where the two liquid layers meet. The alcohol dehydrates and precipitates the DNA, as DNA is insoluble in the alcohol. If the procedure is done properly, fine, long strands of DNA will form at the interface and can be easily spooled onto the glass stir rod.

Disposal

The resulting mixtures can be flushed down the drain according to Flinn Suggested Disposal Method #26b. Please consult your current *Flinn Scientific Chemical & Biological Catalog/Reference Manual* for proper disposal procedures.

Acknowledgment

Special thanks to David A. Katz, Cladwyne, PA for providing Flinn with this activity.

Materials for *DNA Isolation* are available from Flinn Scientific, Inc.

Catalog No.	Description	Price/Each
D0024	Dodecyl Sulfate, Sodium Salt 100 g	Consult Your Current *Flinn Catalog/Reference Manual.*
E0007	Ethyl Alcohol, 500 mL	
GP5075	Glass Stirring Rod	

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Immersion Oils

Publication No. 401.50

Introduction:

Compound microscope objectives in the 90X to 100X range require immersion oil. What does immersion oil do, and what is the proper technique for using it?

Background:

The upper limit of the resolving power of light microscopes is slightly above 1000X. Objectives of 90 to 100X, when coupled with a 10X eyepiece, approach that upper limit. Even in the range of 900 to 1000X, a clear image is only possible if every bit of available light is directed through the microscope optics to the viewer's eye. Immersion oils play an essential role in maximizing the amount of light producing the image the viewer sees.

In the airspace between the slide and the objective lens, light is refracted, scattered, and effectively lost. This happens because the refractive index of air (approximately 1.0) is very different from that of glass (approximately 1.5), and light passing through a glass/air interface is refracted (bent) to a large degree. By reducing the amount of refraction at this point, more of the light passing through the slide will be directed to the very narrow diameter lens of the high-power objective. The more light, the clearer the image. Placing a material with a refractive index equal to that of glass in the airspace between slide and objective directs more light through the objective and produces a clearer image. Immersion oils are formulated for just this purpose.

High-power objectives of 90X or higher are almost invariably intended for use with oil and will be engraved with the words, "oil", or "immersion", or "HI" (homogeneous immersion). These objectives are assembled with special sealants that prevent penetration of oil into the lens system. *Applying oil to an objective not designed for immersion will ruin the objective.*

Immersion oils are commonly available in two viscosities — low viscosity (Type A), and high viscosity (Type B) — and should be labeled with a refractive index of 1.515. The low viscosity oil is applied to the airspace between slide and objective, the high viscosity oil is (less commonly) applied between the condenser and the slide.

Procedure:

Low viscosity oil between slide and objective:

1. With low- or medium-power objective, locate a point or area of interest on the slide and center it in the image field.
2. Rotate the objective turret so that the high power objective is just to one side of the slide. Place a single drop of immersion oil (low viscosity, Type A) on the slide (using the circle of light from below as a guide) and place a drop directly on the objective lens. Failure to apply oil to the objective will likely result in trapped air and reduced image quality.
3. Slowly rotate the high power objective into place and adjust the fine focus to fully resolve the image.

High viscosity oil between condenser and slide (optional).

Condensers with a numerical aperture (N.A.) of 1.0 and greater (usually engraved directly on the condenser) are also sealed to prevent oil penetration. Do not immerse condensers with an N.A. less than 1.0.

1. Before placing the slide on the microscope stage, rack the condenser down (using the condenser focusing mechanism) and apply a drop of oil (high viscosity, Type B) to the condenser lens.
2. Apply a drop of oil to the bottom of the slide directly below the specimen, and place the slide on the stage so that the drops will meet when the condenser is raised.
3. Raise the condenser until the drops converge. Follow the steps detailed above to oil the slide to the objective.

Cleanup:

Immersion oil should be cleaned from lens and slide surfaces when observations are complete. Oil left on lens surfaces will eventually dry and be very difficult to remove.

1. Carefully wipe oil from all glass surfaces with a folded piece of clean lens paper.
2. With a second piece of lens paper, moistened with a small amount of alcohol (ethyl or isopropyl), wipe glass surfaces to remove any streaks of residual oil.
3. To remove oil that has been allowed to dry on lens surfaces, moisten a folded piece of clean lens paper with a small amount of xylene. Gently wipe lens sufaces, giving the xylene a few moments to work. Xylene may soften cements used to assemble the objective—so wipe the surfaces again with clean lens paper moistened with dilute alcohol or distilled water.

References:

Delly, J. G. *Photography Through the Microscope;* 9th ed., Eastman Kodak Co.: Rochester, NY, 1988; pp 18–19.

Leonard, W. H. *A Practical Guide for Microscope Use and Care;* Swift Instruments, Inc.: San Jose, CA, 1994; pp 15, 23.

Materials are available from Flinn Scientific, Inc.:

Catalog No.	Description	Price/Each
I0051	Immersion Oil, Low Viscosity 0.5 oz.	Consult Your Current *Flinn Catalog/ Reference Manual.*
I0052	Immersion Oil, Low Viscosity 1.0 oz.	
I0053	Immersion Oil, High Viscosity 0.5 oz.	
I0054	Immersion Oil, High Viscosity 1.0 oz.	
AB1175	Lens Paper, 50-sheet book	

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Root Words

Publication No. 10321

Introduction

Biological terminology can be as overwhelming to learn as a foreign language. Having a Root Word List can help in decoding the biological foreign language.

Concepts

- Word derivation

Materials

Greek and Latin Root Word List

Procedure

Copy the list found on the reverse side of this BioFax and distribute for classroom use.

Tips

- Create a list of words and have students derive a "literal-root" definition of the word.

  Example: Microcephalic (small head)

  micro— small

  cephalo— head

- Create a list of definitions and have students derive a word.

  Example: Red blood cell (erythrocyte)

  erythros— red

  cyte— cell

Reference

Kessler, J. W. "An Alternative Approach to Teaching Biological Terminology*"; The American Biology Teacher,* **1999**, *61*, 9.

Greek and Latin Root Words

Root (Source)	Meaning	Example
a, an (G)	without, not	abiotic, anaerobic, asymmetry, atrophy
ambi (L)	on both sides	ambidextrous, ambivalent
amphi (G)	both, doubly	amphibian, amphiblastula
andro (G)	male, masculine	androgen, android, Andros
ante (L)	before, in front of	antedate, anteflexion, anterior
anti (G)	against	antibody, antigen
aqua (L)	water	aqueous, aquifer
archeo (G)	original, ancient	*Archaeopteryx*, archegonium, archeology
arthro (G)	joint	arthritis, arthrology, arthropod
auto (G)	self	autogenous, automatic, autonomic
bi (L)	two, twice, double	bilateral, bipedal, pipinnate, bivalve
bio (G)	life, living	biogenesis, biogeography, biology
cephal, cephalo (L)	head	cephalic, cephalothorax
chromo (G)	color	chromatin, chromosome
cide (L)	killer, killing	hericide, insecticide, pesticide
circum, circa (L)	around, about	circadian, circumference
corpus (L)	body	corpus luteum, corpuscles
crypto (G)	hidden	cryptic, cryptogram, cryptorchidism
cyte, cyto (G)	cell, hollow	cytoplasm, leukocyte
derm, dermis (G)	skin	dermal, Echinodermata, ectoderm
di (G)	two, double	diatom, dihybrid, disaccharide
dys (G)	bad	dysentery, dysfunction, dystrophy
e, ec, ef, ex (L)	out of, from	eccentric, emit, excretion, exit, extinction
ecto (G)	outside of, external	ectocarpus, ectoparasite, ectoplasm
endon, endo (G)	in, internal	endoderm, endopodite, endosperm
epi (G)	upon, above	epidermis, epigenesis, epiphyte
erythros (G)	red	erythrocyte, phycoerythrin
exo (G)	outer, external	exoskeleton, exothermic
gam, gamo (G)	marriage, sexual	gamete, gametophyte, gamogenesis
genesis, genic (L)	origin, birth, producing	carcinogenic, cytogenic, mutagenic
genos, gen, geny (G)	race, kind	genealogy, genetics, genotype
herba (L)	grass, plants	herbaceous, herbal, herbivore
heteros (G)	different, other	heterotrophic, heterosexual, heterozygous
homo (L)	man	hominid, *Homo sapiens*
homos, homeos (G)	alike, same	homogeneous, homologous, homozygous
hydro, hudor (G)	water	hydrology
hyper (G)	above, beyond	hyperactive, hyperglycemia, hypertonic
hypo (G)	below, under	hypodermic, hypoglycemia, hypotonic
intra (L)	within, inside	intracellular
intro (L)	into, within	introversion
itis (G)	disease, inflammation	appendicitis, arthritis, hepatitis
logos, logy (G)	the study of	biology, zoology
lys, lysis (L)	dissolution, destruction	hemolysis, hydrolysis, lysosome
mikros, micro (G)	small	microbe, microscope, microspore
monos (G)	one, single	monocular, monocotyledon, monoecious
morphe (G)	form, shape	ectomorph, lagomorph, morphology
neuro (G)	nerve	neurology, neuromuscular, neuron
oo (G)	beginning, egg	oocyte, oogenesis
ovum, ovi (L)	egg	ovary, oviduct, ovipositor, ovule
para (G)	beside, near	paramedic, paranoid, parasite
pedi (G)	beginning, child	pediatrician, pediatrics, pedigree
pes, pedis, pod (L)	foot	bipedal, pedal
photo, phos, phot (G)	light	photosynthesis
post (L)	after, behind	posterior, postnatal, postpartum
semi (L)	half	semicircle, semilunar
skleros (G)	hard	sclera, *Scleria*, sclerenchyma, sclerotic
soma (G)	body	centrosome, lysosome, somatic, somite
sub, sup (L)	below, under, smaller than	subspecies, supination
super (L)	above, upper	supercerebral, superior
supra (L)	above, upon	supraorbital, suprarenal
tetra (L)	four	tetrapod, tetraspore
tox, toxikon (G)	poison	antitoxin, toxic, toxin
tri (L)	three	triploid, tripod
trophe (G)	to feed or eat	autotroph, dystrophy, heterotroph
uni, unis (L)	one	unicellular, uniform, united
xeros (G)	dry	xeric, xerophyte
zo, zoon (G)	animal	holozoic, protozoa, zoology, zoospore

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